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Alkaline phosphatase activity of the epidermoid carcinoma of the portio and its changes due to the γ rays of Radium

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ALKALINE PHOSPHATASE ACTIVITY OF THE EPIDERMOID CARCINOMA OF THE PORTIO AND ITS CHANGES DUE TO THE γ-RAYS OF RADIUM*

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Recently we have reported histochemical changes observed in the organs of rats killed with a lethal dose of X rays [18, 19]. In this paper we shall discuss the effect γ-rays of radium exert on the epidermoid cancer of the portio as far as phosphatase activity is concerned.

Phosphatase is an enzyme splitting off phosphoric esters. It is found in animals and vegetables alike. It participates in various metabolic processes and its role is stressed by the fact that it plays a prominent part in the lecithin and nucleinic acid molecules of the organism. The important correlation of phosphatase and nucleinic acids has been pointed out by numerous authors.

Before discussing these correlations, the morphologic changes occurring 10 to 16 days after the γ-irradiation of the epidermoid cancer of the portio should be briefly summarized [6, 16]. In the stroma there is a numerical increase of fibres of connective tissue, further an extensive plasmocellular infiltration. The augmented stroma invades the tumour and this latter is divided into small groups of cells or single cells. The signs of the ray effect mainly appear in the nuclei (pycnosis, vacuoles, degeneration of nuclei, deformation of the nucleoli, formation of giant cells, chromatosis of the cell membrane, nucleolar destruction, incomplete mitoses, etc.) they may, however, also be observed in the protoplasma (vacuoles, marked acidophilia). Further, acidophilic cells without nuclei are frequently found.

The greater radiosensitivity of the nuclei has been demonstrated by both morphology, and by experiments [23]. The nucleus of an ovum irradiated with a strong dose from 40 000 to 50 000 r is destroyed, nevertheless its plasma may still undergo fertilisation and mitosis after two days.

Up to now 51 cases of portio cancer have been studied. In 33 of these cases excision for histology was done at least twice i. e. before and several days (about 10 to 14) after irradiation. 6 cancers were examined histologically

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only before, and 5 cases only after irradiation. In 7 cases the specimen proved unfit for further histology or histochemical examination. The tubes employed contained 10 mg of radium element within a platinum filter 1 mm thick and were placed into a gold capsule equivalent to a 1 mm platinum filter. In the cases examined twice the first excision was done immediately before application of the tube and the second after 10 to 14 days. The tissue was fixed in 85 per cent chilled alcohol and treated with alcohol-benzole, and paraffin. The tissue pieces obtained from the same patient at various times were mounted on one slide and subsequently Gömöri's alkaline phosphatase reaction [5, 9, 10] was performed on them. The substance to split was

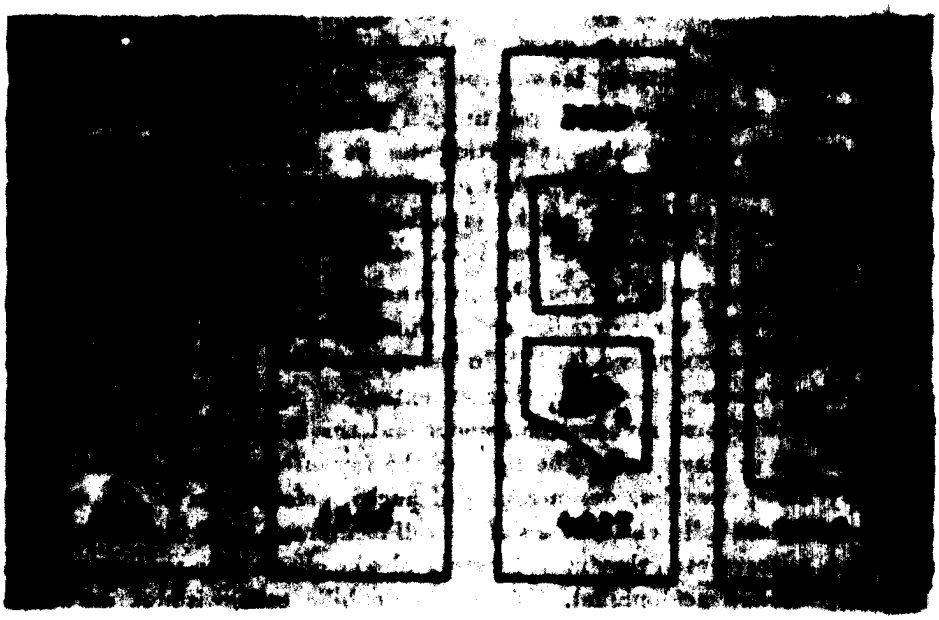

Fig. 1

sodium-β-glycerophosphate, the buffer was veronal sodium. The specimens were incubated from 20 to 24 hours. The precipitated calcium phosphate was rendered visible by Kossa's reaction.

In these specimens, the effect of irradiation manifesting itself in a greater phosphatase activity was recognised already on inspection (Fig. 1).

Some of our cases should be reported in detail:

Fig. 1a

No. 1950) *before irradiation* — section made up chiefly of vascular tissue. The parenchyma consists of confluent cancer nests composed of partly spinocellular partly transitional cells. Enzyme activity seen only in the vessel wall. (Zeiss optics. Objective 10 x. Ocular 4 x)

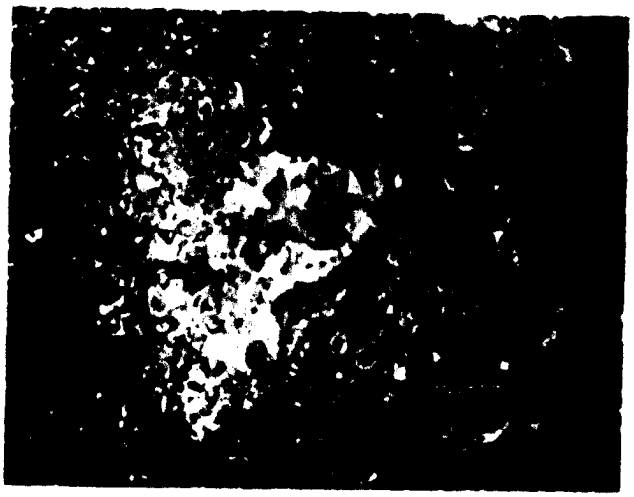

Fig. 1b

(No. 1938) 11 days *after irradiation* with 1840 mgh Ra — granulation tissue with marked phosphatase activity within which some isolated tumour cells or cell groups exhibiting ray effect are seen. The tumour cells are phosphatase negative while phosphatase positivity in the surrounding tissue is considerable. (Zeiss optics. Objective 10 x. Ocular 4 x)

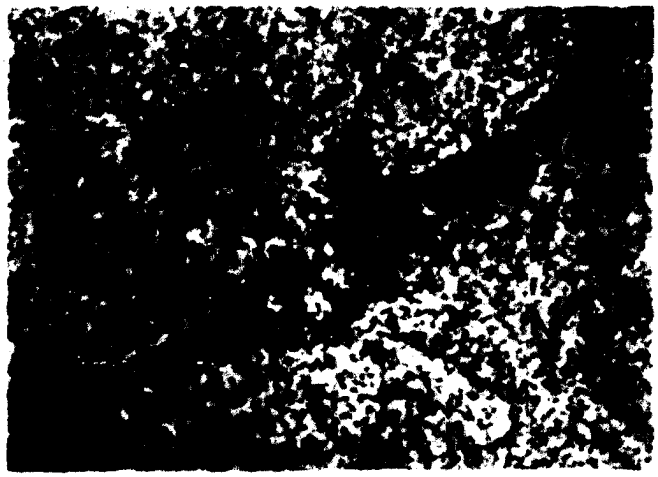

Fig. 2a

(No. 5124). *before irradiation* partly spino-cellular partly transitional cell- forming **confluent** nests **of epidermoid cancer. Scanty stroma** hardly recognisable by small vessels **Activity only in the vessel walls.** (Zeiss optics Objective 8 x Ocular 4 x)

Fig. 2b

(No. 5216). 16 days *after irradiation* with 1840 mgh Ra granulation **with high phosphatase** activity Necrotic tissues and tumor cell groups are negative. There is a **striking enzyme** activity in the environment of the rest of the tumour nests (Zeiss optics Objective 8 x Ocular 4 x)

Patient 3 M. L. 75 years old.

Fig. 3a
(No. 5864). *before irradiation* moderately developed loose stroma with interlacing epithelial bundles the cells of which closely resemble basal cells. Activity in the vessel walls only.
(Zeiss optics. Objective 10 x. Ocular 4 x)

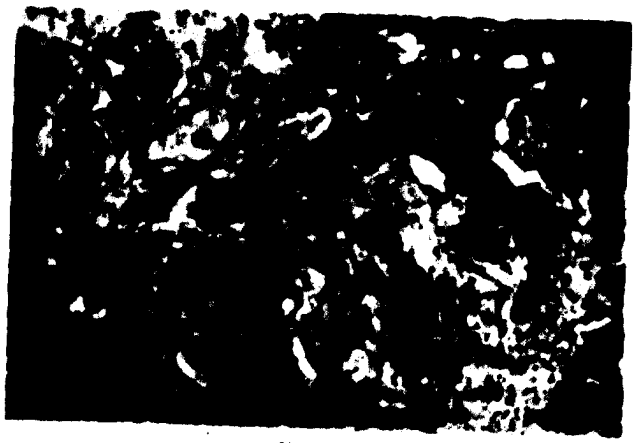

Fig. 3b
(No. 5380). 14 days *after irradiation* with 1250 mgh Ra. The tumour proved rather resistant. Increased stroma showing considerable enzyme activity
(Zeiss optics, Objective 10 x. Ocular 4 x)

Similar results were obtained also in the other patients. Strikingly, enzyme activity was present only in those tumour nests which had a basocellular character and could be regarded as radiosensitive (2 cases). In other cases the tumour showing a basocellular character was phosphatase negative and, simultaneously, rather resistant to irradiation. The ratio of spinocellular, basocellular, and transitional cell tumours should not be dealt with here, these terms being frequently subject to individual judgment. The enzyme activity of the stroma

was varying whereas the endothelial lining of the vessels displayed phosphatase positivity in nearly all cases.

Irradiation resulted in granulation of the tissue which was highly active aside from the remainder of tumour cells seated in the granulation; the tumour cells were invariably negative. The activity appeared in the new fibrous tissue, the endothelial lining of the vessels, the plasma cells and the fibroblasts. It was striking that there was a greater activity in superficial layers more exposed to ray effect than in the deeper layers, further a greater activity appeared in the close neighbourhood of the tumour cells than in other places. No activity was observed in the necrotic areas, in the giant cells of foreign body type which pointed to absorption difficulties in the media of the vessels and eventually in some plasma cells.

Our results cannot be evaluated without a few literary data.

Darlington [4] has observed that the poverty of cells in nucleinic acids is associated with the incomplete division of chromosomes; the latter cannot detach themselves from one another and thus a chromatine bridge arises and remains after the cell division. Similar incomplete mitoses were seen in the irradiated tissues.

Errera [7, 8] states that irradiation is accompanied by depolymerisation of thymonucleohiston.

Holmes [13] performed experiments with Jensen's rat sarcoma. He injected the animals with isotope P_{32} and found that irradiation resulted in the reduction of metabolism as the assimilation of P_{32} by the thymonuclein fraction was reduced. These results are in accordance with some earlier observations of *Hevesy* [11] who demonstrated that, in animals suffering from two tumours, the irradiation of one tumour resulted in diminished assimilation of P_{32} by the other.

Our own examinations [18, 19] showed that phosphatase activity in the liver of rats killed with lethal irradiation was very considerable while no activity occurred in the liver of control animals.

Hevesy [12] found some correlation between the thymonucleinic acid metabolism of cell nuclei and alkaline phosphatase.

Caspersson and *Thorell* [2] claim that, during embryonic development, phosphatase activity changes parallel with the concentration of nucleinic acids and nucleoproteids, respectively. This enzyme is likely to have a role in the assimilation and dissimilation of nucleinic acids.

Krugelis, Danielli and *Catcheside* [3] examined the activity of alkaline phosphatase in the cells of drosophilae. The active areas showed a formation resembling wood-work in the chromosomes. This formation corresponds with the »Feulgen-positive« strands.

Vickerson, Krugelis and *Andresen* [17] found in yeast fungi, provided that sodium glycerophosphate was used as a matter to split, the site of phos-

phatase activity in a dark spot of the cell, resembling a Feulgen positive stain. The morphologic existence of this spot was proved by examination with phase contrast microscope.

Bradfield [1] demonstrated high phosphatase activity in the nucleus of cells lining the silk glands of spiders and in the corresponding cells of the caterpillar of a moth, in parts of the cells bordering the surface of the lumen, further in the excreted discharge.

Jeener [14] examined the phosphatase activity in ovarectomized mice following oestradiol administration. The activity was greatly increased in the proliferating vaginal wall and the uterus, especially in the circular muscle layer of the latter.

The two authors last quoted, as well as several others, contend that the increase of phosphatase activity may be related to the formation of fibrous protein (in the latter case: keratine, myosine).

Similar results have been obtained by *G. Vargha* [22] with productive tuberculous changes of lung tissue.

Kate, Spein and *Ljob* observed that the closure of wounds takes comparatively time if the wound surface is smaller. They observed further that cells and nuclei begin to grow after the injury, attaining a maximum size at the time of wound closure, after which they shrink again. *Olga Lepeshinskaya* inferred from this fact [16] that the products of cell destruction played a great role in the nutrition of tissues and the stimulation of growth and cell proliferation. It is possible that these living substances are adapted in the formation of new cells. Following these conclusions Lepeshinskaya examined the role of blood effusion in wound healing and stated that the products of blood destruction considerably promoted the process of repair.

In this institute, *Redé* [20, 21] performed successful experiments treating torpid wounds with irradiated blood.

On the basis of our examinations and the data of literature it seems to be warranted that irradiation results in depolymerization of the nucleoproteids contained in the nuclei of tumour cells and other tissue elements on account of which the nucleoproteid becomes soluble. This phenomenon manifests itself in the activity of alkaline phosphatase which after irradiation gives rise to new formation of a fibrous connective tissue. This theory is in accordance with the fact that cellular and less differentiated tumors are more radiosensitive, and also with the observation that the superficial layer of the granulation tissue having been more exposed to ray action, shows a higher enzyme activity than the deeper layers. Finally the enzyme activity is also stronger in the near environment of the remnants of the tumour than in other places.

Further histochemical and other examinations are being performed to support these assumptions.

The effect of roentgen and γ-rays on living tissues and tumours was studied. In the first line the changes of enzyme activity were examined by means of well-known histochemical methods. In this paper the action of the γ rays of radium on the alkaline phosphatase contained in the epidermoid cancer of the portio was examined.

The excised material of 51 cancerous portios was examined. In 33 of 51 cases excision was done at least twice, before and after irradiation (10 to 11 days later on an average). The excised tissues taken from the same patient were mounted to the same slide. Gomori's method was applied to the specimens. The considerable increase of enzyme activity following irradiation could in the majority of cases be seen on gross inspection. The tumor parenchyma was invariably phosphatase negative before irradiation, apart from 2 absolutely radiosensitive cases. The phosphatase activity of the stroma was varying but the endothelial cells of the vessels showed phosphatase activity in almost every case. 10 to 11 days after the irradiation granulation tissue appeared with great phosphatase activity in the new connective tissue fibres, vessel walls, fibroblasts and plasma cells. No activity was shown by the remaining tumour cells, necrotic areas, foreign body giant cells, vessel media, and some plasma cells. Strikingly greater enzyme activity was found in superficial tissue layers more exposed to the rays, further in the close neighbourhood of the remainder of tumour, also in the deep layers or farther from the tumour cells.

Both our examinations and the literary data seem to support the assumption that irradiation results in depolymerization of the nucleoprotein of tumour cells and other tissue elements whereby it becomes soluble in the tissue fluid. This process manifests itself in phosphatase activity which gives rise to the formation of new connective tissue after irradiation.

We thank Zsanna Bélint-Huppert and Karola Hafner, technical assistants, for their valuable cooperation.

REFERENCES

1. *Bradfield, J. R. G.* (1946) Alkaline Phosphatase in Invertebral Sites of Protein Secretion. Nature 157. 876.

2. *Casperssen, T. Thorell, B.*: (1940) Chromosoma II. 132. Quoted by H. Baur (1948—49) Tierische Phosphatasen. Z. Vit.-Hormon- u. Fermentforschung II. 560.

3. *Danielli, J. F. Cascheside, D. G.* (1945) Nature 156. 294. Quoted by H. Baur (1948-49): Tierische Phosphatasen. Z. Vit.-Horm.- u. Fermentf. II. 566.

4. *Darlington*. Quoted by *Lajtha L.* (1948). A nucleinsavakról. Orvosok Lapja 50. 1589.

5. *Kabat, E. A. Furth, J.* (1941) A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. Am. J. Path. XVII. 308.

6. *Engelmann* (1938). Die mikroskopischen Veranderungen an den Tumorzellen und den gesunden Geweben des Menschen nach Strahlenbehandlung. Erschienen in Holfelders Röntgentiefentherapie. (G. Thieme, Leipzig.)

7. *Errera, M.* (1946) Action des rayons X sur le nucleolonion d'erythrocyte de poule in situ. Compt. Rend. Soc. Biol. 140.

8. *Errera, M. Bruchet J.* (1946): Action des rayons X sur le thymonucleolonion d'erythrocyte de poule in situ. Compt. Rend. Soc. Biol. 143.

9. *Gomöri* (1939): Microtechnical demonstration of phosphatase in tissue sections. Proc. Soc. Exp. Biol. Med. 42. 1.

10. *Gomöri* (1941) The distribution of phosphatase in normal organs and tissues. J. of Cell. Comp. Phys. 17. 71.

11. *Heaney* (1945): Rev. Mod. Physics. XII. 102. Quoted by Lajtha (v. No. 4).

12. *Heaney* (1948) Quoted by Lajtha, ibidem.

13. *Holmes, B. E.* (1947): The inhibition of thymo- and ribonucleic acid synthesis in tumour tissue by irradiation with X rays. Brit. J. Radiol. XX. 249. 450.

14. *Jenner, R.* (1947) Cytochemical effects of oestradiol. Nature. 159. 578.

15. *Lahm, W.* (1928) Untersuchungen an fortlaufenden Probeexcisionen über biologische Strahlenwirkungen u. Carcinomheilung. Strahlentherapie 27. 642.

16. Лепешинская О. Б.: (1950) Происхождение клеток из живого вещества и роль живого вещества в организме. Издательство Академии медицинских Наук СССР. 304. стр.

17. *Nickerson, W. J. Krugulis E. J. Andresen, N.* (1948) Localisation of phosphatase in yeast. Nature 162. 192.

18–19. *Rée, K., Rée, L., Lénárt, [illegible]* (1951) Röntgensugarakkal ölött patkányok [illegible] alkalikus phosphatase-aktivitás változás. Kísérletes Orvostudomány III. 6. 444. (1952) Alkaline Phosphatase Activity-Changes in Organs of Rats killed with Roentgen-Rays. [illegible] Morphologica I. 4. 467.

20. *Radó, I.:* (1948) Az effektagen therápia. [illegible] Orvosok Lapja.

21. *Radó, I.:* (1951) Az in vitro [illegible] Orvosi Hetilap.

22. *Varghа, G.:* (1950) A [illegible] phosphatase-aktivitás [illegible]. Kísérletes Orvostud. II. 4. 290.

23. *Whiting, A. R.:* (1946) Motherless males from irradiated eggs. Science 103. 219.

[Ак]тивность щелочной фосфатазы плоскоклеточного рака влагалищной порции матки и изменения этой активности под действием лучей γ радия

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В нашем институте мы исследовали действие рентгеновских лучей и лучей «γ» [н]а живые ткани и на опухоли. В первую очередь мы исследовали с помощью [ги]стохимических реакций изменения ферментативной активности. В настоящем сообщении мы останавливаемся на действии лучей «γ» радия на плоскоклеточный рак влагалищной порции матки, с точки зрения активности щелочной фосфатазы.

Мы обработали материал, полученный от 51 больных, страдающих раком шейки. [Ср]еди этих мы проводили в 23 случаях два раза пробную эксцизию, в среднем 10–14 дней [д]о и 10–14 дней после лечения лучами. Полученные таким образом материалы мы наклеивали на общее предметное стекло и там провели реакцию определения активности щелочной фосфатазы по Гомори. В большинстве случаев уже не вооруженным глазом можно было отметить значительное повышение активности щелочной фосфатазы под влиянием [облу]чения лучами. Во всех наших случаях до облучения первичная опухоль с точки зрения [ак]тивности щелочной фосфатазы давала отрицательную реакцию за исключением двух случаев. Но в этих случаях оказалось, что данные опухоли чрезвычайно чувствительны к лучам. [Ак]тивность фосфатазы в строме колебалась, однако эндотелиальные клетки сосудистой [ст]енки везде давали положительную реакцию. Под действием облучения в течение 10–14 [дн]ей развилась грануляционная ткань, с выраженной активностью фосфатазы. В этой [ткан]и самая высокая ферментативная активность наблюдалась в новообразованной [мол]одой соединительной ткани в стенке сосудов, во фибробластах и в [illegible] [кле]тках.

Оставшиеся опухолевые клетки, частицы некротической ткани, гигантские клетки [ти]па гигантских клеток появляющихся возле инородных тел, указывающие на то, что [зде]сь резорбция происходит медленно, интима артерий и вен, а кое-где единичные плазмоцитарные клетки дали отрицательную реакцию на активность фосфатазы. Выяснилось [так]же, что в более поверхностных слоях [illegible] лежащих ближе к радию, активность фермента являлась более высокой, чем в более глубоких слоях. Наше внимание привлекло [и] то что вокруг единичных опухолевых клеток мы наблюдали более высокую активность [фос]фатазы чем на других местах.

Результаты наших исследований и имеющиеся в нашем распоряжении литературные данные говорят за то, что под действием лучей нуклеопротеиды ядер опухолевых клеток а также ядер других тканевых элементов деполимеризуются и становят растворимыми в тканевой жидкости. Это отражается в проявлении реакции фосфатазы и это вызывает прогрессирующий вклад за облучением новообразование молодой соединительной ткани.

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